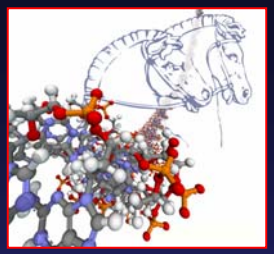

Green Fluorescent Protein Expression in Bone Marrow-Derived Mesenchymal Stem Cells of Immunocompetent and Athymic Nude Rats

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Introduction

Concerns exist regarding potential differences in transgene product expression following gene delivery in immunocompetent versus immunodeficient animal models. To address this question *in vitro*, this study evaluated expression of the reporter gene product green fluorescent protein (GFP) in bone marrow-derived mesenchymal stem cells (BMDMSC) from immunocompetent and athymic nude rats following GFP gene delivery using a first-generation adenoviral vector (Ad). While BMDMSC hold promise as multipotent gene delivery targets, a consensus regarding an ideal carrier has not been reached. It is also essential that the efficiency of new gene delivery systems be validated by the use of reporter genes. Efficient delivery of genes has been achieved using adenoviral vectors.[1,2] This vector has a high transduction efficiency, is easy to propagate with high titers at reasonable cost, and it can transduce both dividing and quiescent cells[3]. The purpose of our study was to use an Ad vector to transduce BMDMSC from rats with GFP *in vitro*, and to evaluate and compare GFP expression in BMDMSC from immunocompetent and athymic nude (i.e. immunodeficient) rats in both monolayer and three-dimensional (3D) alginate cultures. Our hypothesis was that GFP expression in BMDMSC from athymic nude rats and those from Wistar (immunocompetent) rats would not differ in intensity or duration of transgene expression. Our specific goal was to evaluate GFP expression by documenting duration and intensity of fluorescence detected in cells by imaging of transgene product in live cells.

Methods

Bone marrow derived mesenchymal stem cells were harvested from 2-4-week old Wistar (Wtr) rats and 10-week-old NIH-rnu nude rats (Nude) and BMDMSC from Lewis rats (Tul) were obtained from the Tulane Center for Gene Therapy.[a] *Monolayer:* Cells were expanded in monolayer culture and placed in 48-well clear plates with 1000 cells per well when seeded at Day -1 (Day 0 = day of transduction). Cells were transduced for 2 hours with AdGFP. Fluorescence was quantified in live cells using an *in vivo* imaging system (IVIS®) at days 0, 7,12, 22. *Alginate:* Cells were expanded in monolayer culture in 75mm^2 plates and half of the plates were transduced with AdGFP. All cells were suspended in 1 ml of 1.2% sodium alginate at 4.4 x 10^4 cells/ml. Fluorescence was measured using IVIS ® at days 0, 7, 12, and 22.

This work was supported by NIH grant No. F32AR050916 (TAZ), K08AR049201 (ALB) and the Trueman Family Endowment (ALB). The authors thank David Moon and Marc E. Hardman for technical support. [a]Some of the materials employed in this work were provided by the Tulane Center for Gene Therapy through a grant from NCRR of the NIH, Grant No. P40RR017447.

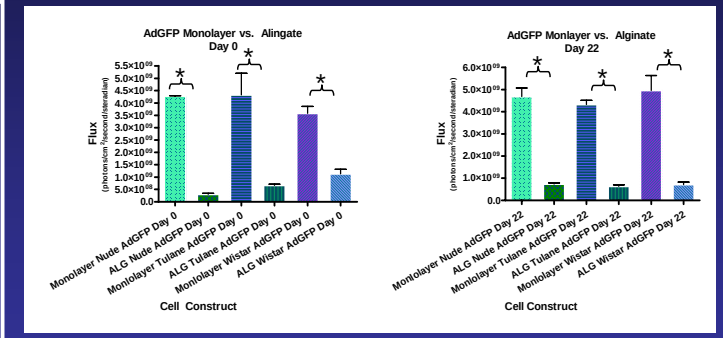

Fig 1. Fluorescence, a measure of green fluorescent protein expression, measured as flux (photons/square cm/second/steradian) detected in monolayer and alginate constructs at Day 0 and Day 22 of the transduced groups (AdGFP) and the control (NoAd). Bars represent median and range.

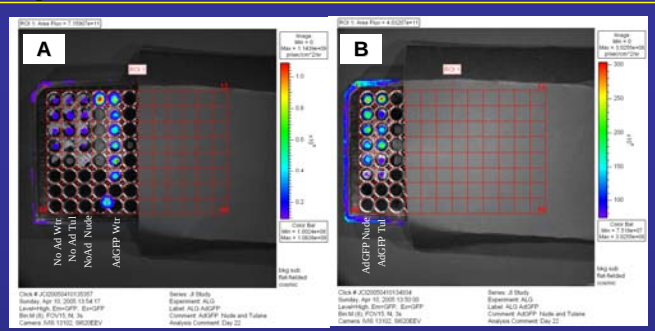

Fig 2. Images of live cell-alginate constructs at Day 20 obtained with an *in vivo* imaging system (IVIS®). **A.** Fluorescence in the NoAd groups is consistent with background fluorescence, whereas that in the AdGFP-transduced cells is significantly greater (P =0.0095). **B.** Expression in AdGFP-transduced cells suspended in alginate is shown for two different cell types.

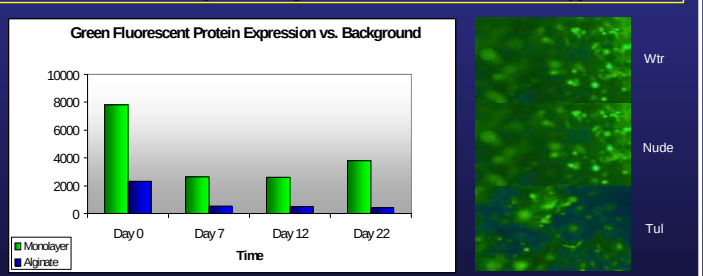

Fig 3. Flux values expressed as a ratio of median expression in AdGFP-transduced cells to background levels.

Fig 4. Live BMDMSC in alginate constructs as seen with fluorescence microscopy. Fluorescent microscopic appearance did not differ among groups.

Statistical Analysis: Expression between groups was compared using Mann-Whitney tests for each time point. A Kruskal-Wallis and Dunn's post test for nonparametric multiple comparisons was performed to determine the difference in GFP expression among the three different cell types, Nude, Tulane, and Wstr cells over time. Significance was defined as a P-value of <0.05. Individual tests were run for controls (i.e. untransduced) and AdGFP-transduced cells in both monolayers and alginate constructs.

Results & Discussion

There were no significant differences in GFP expression between groups (immunocompetent *versus* immunocompromized). Rat breed and immune status had no effect on GFP expression (P = 0.085, monolayer; P=0.5059). Flux values were expressed as a ratio of median expression in AdGFP-transduced cells to background levels (Figure 3) with a range of 3.58x109 - 4.33x109 monolayer, and 2.58x108 – 1.44x109 alginate construct. Background flux was 2.50 x 10^6 for cells in monolayer culture and 1.42 x 10^6 for cells in alginate constructs. The AdGFP-transduced cells had 1800-fold greater expression, when compared with background values, in monolayer culture, and 525-fold greater expression in alginate constructs. Cells cultured in monolayer showed significantly greater expression compared to those in alginate constructs ($P < 0.05$). These findings provide evidence to justify the use of cells from both immunocompetent and immunodeficient animals as gene delivery targets in both *in vitro* and *in vivo* studies. In addition, we confirmed that expression of a fluorescent reporter gene is more readily detected in monolayer cultures than in 3D cultures. This may have implications for the design of *in vivo* models.

Conclusion

We conclude that in this *in vitro* model of transgene expression in monolayer and three-dimensional alginate cultures, there is no difference in transgene expression between cells from immunocompetent animals and those of from immunodeficient nude rats.

References: 1) Lecanda F. *J Cell Biochem* 1997; 2) Liu Q. *Gene Therapy* 2003; 3) Bertone AL. *J Orthop Res* 2004